REMARKS

The Examiner is respectfully requested to provide a Notice of References Cited Form PTO-892, which lists Clive et al., which was applied in a prior art rejection in the April 25, 2001 Office Action.

The presently claimed invention concerns a method of analyzing a target nucleic acid by applying a nucleic acid amplification reaction to a test solution, wherein an amplified product is labeled with a marker molecule. The method comprises:

(a) performing a nucleic acid amplification reaction of the target nucleic acid in a test solution containing a forward primer and a reverse primer, a substrate comprising nucleotides, a nucleic acid polymerase and a target nucleic acid, wherein **the number of one of the forward primer and the reverse primer is lower than that of the other primer, and the primer present in a lower number is labeled with a marker molecule capable of generating a detectable signal to form a labeled primer;**

(b) measuring a signal from the marker molecule in the test solution after initiation of the nucleic acid amplification reaction;

(c) evaluating a fluctuation motion of the amplified nucleic acid which is labeled with the marker molecule, in the test solution on the basis of the signal detected; and

(d) quantifying the target nucleic acid on the basis of evaluation results.

Important features of the present invention reside in that the number of the forward primer or the reverse primer is lower than that of the other primer, and the primer present in a lower number is labeled with a marker molecule capable of generating a detectable signal. According to applicant's method, an amplified double stranded nucleic acid product is labeled with a marker molecule without fail, whereas a single-stranded nucleic acid product is not labeled (see Fig. 3 of the present application). The reaction can be observed by fluorescence correlation spectroscopy ("FCS"). In this manner, the presently claimed invention makes it possible to analyze a target nucleic acid.

Claims 1 to 5, 7, 8, 39 and 42 to 43 were rejected under 35 USC 103 as being unpatentable over Salituro et al. USP 6,391,544 in view of Eigen et al. USP 5,807,677 for the reasons set forth in Paragraph No. 4 on pages 2 to 4 of the Office Action.

It was admitted in the Office Action that Salituro et al. do not disclose any of (i) evaluating a fluctuation motion of the amplified nucleic acid, (ii) quantifying the target nucleic acid and (iii) the specific ratio of the concentration of the primers as recited in applicant's claims 42 and 43.

Eigen et al., in column 3, lines 46 to 57, discuss the advantages of FCS over the polymerase chain reaction ("PCR"). However, **Eigen et al. do not teach or suggest combining FCS with PCR.**

Salituro et al. do not disclose the same asymmetric PCR as in the presently claimed invention. Claim 1 of Salituro et al. recites the following:

> "wherein the improvement comprises providing the first primer sequence in 15% to 250% excess over the second primer and wherein a probe is hybridized to the amplification product from the first primer to form a hybrid complex, and the hybrid complex is detected as an indication of the presence of the target sequence in the test sample" (emphasis supplied).

More specifically, the asymmetric PCR of Salituro et al. is carried out similarly to the asymmetric PCR of Gyllensten et al. (cited in the previous Office Action). In this method, a double-

stranded DNA and a single-stranded DNA are produced by PCR amplification. Only the single-stranded DNA is then detected by a probe.

Furthermore, Salituro et al. describe the following in column 10, lines 5 to 10:

> "Only when the unlabeled primer was present at concentrations over than the labeled primer was a more linear signal produced correlating with target concentration".

As is apparent from above, in Salituro et al., the first primer present in a larger amount is labeled. In contrast thereto, in the presently claimed invention, a primer present in a lower amount is labeled. Hence, the asymmetric PCR of Salituro et al. is completely opposite to that of the presently claimed invention.

In the method of the presently claimed invention, only when the amount of labeled primer is lower than that of unlabeled primer, the phenomenon where free-labeled primer is consumed with the progress of a PCR amplification reaction is observed. Thus, even if the primer which will never be consumed is labeled, significant data for the Salituro et al. method cannot be obtained.

In the present invention, the amount of target nucleic acid contained in a sample before the PCR amplification can be determined based on the number of PCR cycles carried out, until the labeled primer is completely consumed (see FIG. 3 and page 29, lines 1 to 5 of the present specification). This is a specific feature of the presently claimed invention.

In summary, in contrast to the presently claimed invention, Salituro et al. disclose an asymmetric PCR where a primer present in a larger amount is labeled.

It is respectfully submitted that one of ordinary skill in the art would not consider to combine Eigen et al. and Saituro et al., since Eigen et al. do not teach to combine FCS and PCR.

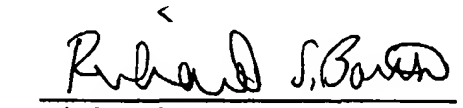
Even assuming *arguendo* that the references are combinable, for the reasons discussed above, it is respectfully submitted that one of ordinary skill in the art would not arrive at the presently claimed invention from the disclosures of the cited references. Stated differently, if one of ordinary skill considered to combine the FCS of Eigen et al. with the PCR of Salituro et al., the presently claimed invention would not be reached because the PCR of Salituro et al. is substantially different from the PCR in the presently claimed invention.

It is therefore respectfully admitted that applicant's claimed invention is not rendered obvious over the references, either singly or combined in the manner relied upon in the Office Action in view of the distinctions discussed hereinabove. It is further submitted that there are no teachings in the references to combine them in the manner relied upon in the Office Action.

Reconsideration is requested. Allowance is solicited.

If the Examiner has any comments, questions, objections or recommendations, the Examiner is invited to telephone the undersigned at the telephone number given below for prompt action.

Respectfully submitted,



Richard S. Barth
Reg. No. 28,180

Frishauf, Holtz, Goodman
& Chick, P.C.
767 Third Ave., 25th Floor
New York, NY 10017-2023
Tel. Nos. (212) 319-4900
(212) 319-4551/Ext. 219
Fax No.: (212) 319-5101
E-Mail Address: BARTH@FHGC-LAW.COM
RSB/ddf

Enc.: PETITION FOR EXTENSION OF TIME